Parasitol Res (2000) 86: 783–786

ORIGINAL PAPER

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The taxonomic importance of obligate heteroxeny: distinction of *Hammondia hammondi* from *Toxoplasma gondii* – another opinion

Received: 27 March 2000 / Accepted: 20 April 2000

Abstract We enumerate identical and divergent findings concerning the obligate heteroxenous *Hammondia hammondi* and the facultatively homoxenous or heteroxenous *Toxoplasma gondii*. Differences exist in life-cycles, transmission, and host range, especially transmissibility to birds and mammals other than rodents, in ultrastructural morphology, immunity and serology in cats and to lesser degree in rodents, in DNA sequences and in isoenzymes. Because the recognition of obligate heteroxeny is essential to study these organisms and to recognize them as taxa, it is advantageous to give heteroxeny a generic rather than a specific value. Characterization of organisms with the life-cycle patterns of *Hammondia*, *Sarcocystis, Frenkelia*, and *Toxoplasma* is best achieved by means of the genera presently used.

Introduction

In the course of a discussion on whether *Neospora caninum* and *Hammondia heydorni* are distinct species, Mehlhorn and Heydorn (2000) posed the question as to whether an obligatory heteroxenous life-cycle was sufficient to establish a new genus. They implied that it was not, and that obligate heteroxeny was a "minor difference in biological processes." The proposal was made to regard *H. hammondi* as a less virulent strain of (*Isospora*) *Toxoplasma gondii*, and *H. heydorni* as an (*Isospora*) *T. heydorni*. The relationships between *H. heydorni* and *N. caninum* will be discussed in a separate paper.

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A number of investigators have confirmed the existence of obligate heteroxenous *Hammondia* spp., whereas *T. gondii* is facultatively heteroxenous. In fact, cats can be called a complete host of *Toxoplasma* (Frenkel 1977), because they support the multiplication of tachyzoites, bradyzoites (Frenkel 1973b), and the gametogonic stages (Dubey and Frenkel 1972). However, cats are only the final host of *H. hammondi*, because only enteroepithelial stages are formed.

H. hammondi has been isolated from the continental United States (Frenkel and Dubey 1975a), Hawaii (Wallace 1975), Germany (Rommel et al. 1976), Switzerland (Ellis et al. 1999), Australia (Mason 1978), and Japan (Shimura and Ito 1987). In addition *H. pardalis* was described from an ocelot in Panama with oocysts averaging 28.5×40.4 μm, whereas those of *H. hammondi* average only 10.6×11.4 μm (Hendricks et al. 1979).

It is likely that a considerable amount of evolutionary time was required for the trait of obligate heteroxeny of *H. hammondi* to evolve from the facultative heteroxeny of the ancestral *Toxoplasma*. With *H. hammondi* neither tachyzoites nor bradyzoites from rodents are transmissible to other rodents, nor can sporozoites from oocysts infect other cats. The trait of transmission between intermediate hosts or between final hosts may have been lost because heteroxenous transmission was more efficient, with cats eating mice more often than ingesting oocysts from soil. Genomic drift in *T. gondii* has been discussed (Frenkel and Ambroise-Thomas 1977).

The loss of transmission between intermediate hosts is paralleled by the limited multiplication of *H. hammondi* in cultured cells, whereas *T. gondii* proliferates indefinitely (Sheffield et al. 1976). Only tachyzoites of *H. hammondi* are formed in mouse embryo, *Rhesus* monkey kidney or the W-38 cell line of human fibroblasts (Sheffield et al. 1976); however, cysts with functional bradyzoites were found in kidney cell cultures from felines, the final host (Riahi et al. 1995). *T. gondii* bradyzoites develop in slowly growing cell cultures of many hosts (Hoff et al. 1977).

Although they are morphologically similar, *H. hammondi* differs from *T. gondii* in three criteria, which were not considered by Mehlhorn and Heydorn (2000). A crystalloid body (Sheffield et al. 1976) is present in sporozoites of *H. hammondi* and *H. heydorni*, which is lacking in *T. gondii* (Speer at al. 1998). Tachyzoites of *H. hammondi* and H. *heydorni* have electron-dense rhoptries, whereas those of *T. gondii* are electron-lucent (Sheffield et al. 1976). *H. hammondi* bradyzoites measure only 4–5 × 1.2 μm whereas bradyzoites of *T. gondii* measure 7–8 × 1.5–2.0 μm (Mehlhorn and Frenkel 1980). In view of these features, *H. hammondi* and *H. heydorni* are structurally different and *H. hammondi* is not "indistinguishable morphologically" from *T. gondii* as believed by Mehlhorn and Heydorn (2000).

Before describing *H. hammondi* as different from *T. gondii* (Frenkel and Dubey 1975a, b), we had unsuccessfully attempted to infect 16 cats, the final host shedding oocysts, with 10^4–10^6 oocysts, without obtaining oocyst shedding. Feeding the tissues from two of these cats to other cats again did not result in oocyst shedding; subsequently showing the cats to be susceptible to infection with *H. hammondi* bradyzoites indicated that they had not been silently immunized. *T. gondii* oocysts would have infected cats, though not efficiently.

However, after feeding *H. hammondi* tissue cysts from mice, we found oocyst shedding in 29 of 30 cats (Frenkel and Dubey 1975a, b). Although Mehlhorn and Heydorn (2000) considered our transmission attempt to be "very scarce", we used 2–4 times as many cats, given the outcome, than would have been necessary to establish statistical significance.

Tissue cysts of *H. hammondi* are formed in rodents, but not in cats, whereas *T. gondii* forms tissue cysts containing bradyzoites in cats. There was no cross-immunity in cats, irrespective of whether *T. gondii* or *H. hammondi* was the first infection, and such cats shed oocysts after each infection; however, cats develop immunity to homologous challenge (Frenkel and Dubey 1975a, b). Wallace (1975) obtained similar findings with a Hawaiian isolate of *H. hammondi*. The minimum prepatent period of cats infected with bradyzoites of *H. hammondi* is 5 days and for *T. gondii* is 3 days (Frenkel and Dubey 1975a, b).

Attempts to infect mice with *H. hammondi* bradyzoites from other mice were unsuccessful, and the carcasses of ten such inoculated mice or hamsters did not elicit oocyst shedding in three cats; these cats were later shown to be susceptible to *H. hammondi* bradyzoites from mice, indicating that they had not been silently immunized. Although mice develop low dye test titers and partial cross-immunity to *T. gondii* after *H. hammondi* infection, 14 cats did not develop antibody or cross-immunity (Frenkel and Dubey 1975a, b; Wallace 1975). Hence, by using cats, *H. hammondi* is not "indistinguishable" from *T. gondii,* based on serological and immunological criteria, as asserted (Mehlhorn and Heydorn 2000).

In mice, *H. hammondi* infection gave rise to cross-reacting *Toxoplasma* antibody in the dye, ELISA and complement-fixation tests, but not in the fluorescent antibody test and the indirect hemagglutination test (Weiland et al. 1979). Infection with *H. hammondi* induced only partial immunity against *T. gondii* in goats (Dubey 1981) and Tammar wallabies (Reddacliff et al. 1993).

Antigenic similarities exist between *H. hammondi* and *T. gondii* (Araujo et al. 1984); however, as these authors emphasize, antigens of similar molecular weight are not necessarily identical, but homologous. Individual monoclonal antibodies directed against the internal organelles of *T. gondii* cross-reacted only weakly with the apical complex, dense granules, micronemes and rhoptries of *H. hammondi*, again indicating similarities, but not identity (Riahi et al. 1999). *H. hammondi* is certainly not "indistinguishable" from *T. gondii* as suggested (Mehlhorn and Heydorn 2000).

Phylogenetic relationships were investigated by DNA sequence comparisons of the D2/D3 domain of the large subunit rDNA and the internal transcribed spacer 1 (ITS1) (Ellis et al. 1999). *H. hammondi* and *T. gondii* form a monophyletic group with unique differences in nucleotide positions 17, 19, 50, 149, 187, 229, 441, and 484 in the sequence alignment of the LSU rDNA, and they share three character states (96, 185, and 230). They differ in 3.2% of the ITS1 nucleotides, a gene that is considered of taxonomic utility (Jenkins et al. 1999).

Isoenzyme analysis was carried out by Dardé et al. (1992), using isoelectric focussing in polyacrylamide gels. All five isoenzymes tested were different in the original isolate of *H. hammondi* and three isolates of *T. gondii* belonging to different zymodemes.

All mammals and birds are typically susceptible to *T. gondii*, as shown by illness, positive subinoculation or a serological response (Miller et al. 1972). Oocysts of *H. hammondi* are infectious to rats, hamsters, *Peromyscus maniculatus*, *Mastomys coucha,* and guinea pigs, but not to four pigeons and two calves (Frenkel and Dubey 1975a, b; Fayer and Frenkel 1979). After inoculation with 10^6 or more oocysts of *H. hammondi*, Wallace (1975) infected 2 dogs, based on antibody responses, but was unable to infect 4 pigeons, 18 Japanese quail, and 2 *Macaca irius*, which remained seronegative. Dubey and Streitel (1976) failed to infect six chickens. Low levels of fluorescent antibodies to *H. hammondi* were found in humans, but except for three instances, they were associated with high anti-*Toxoplasma* titers in the dye test (Wallace 1975). While infection in humans could not be excluded, there was no clear evidence for its presence.

To combine two taxa with different transmission patterns, biologic and morphologic characteristics under the same specific name would conflict with the objective of the Code of Zoological Nomenclature, specifying the name of each taxon should be unique and distinct (Preamble). The nomenclature should be stable (Art. 23-b), and because of the long use of *T. gondii* (91 years) and *H. hammondii* (25 years) there is no need to change to the genus *Isospora*, using *Toxoplasma* as a subgenus, and in effect creating a trinomial nomenclature. This had already been proposed by Overdulve (1970) but it has not found general acceptance.

The finding of similar morphologic characteristics in *H. hammondi* and *T. gondii*, as stressed by Mehlhorn and Heydorn (2000), does not diminish the taxonomic value of divergent characteristics. Identifying a facultative heteroxenous life-cycle in *T. gondii* between 1965 and 1970 (reviewed in Frenkel 1973a, b) with unique developmental stages in the gut of the final host, initiated a systematic search for other heteroxenous cycles in tissue-cyst-forming Protozoa. Indeed, heteroxenous life-cycles were identified for *Sarcocystis, Frenkelia*, *Besnoitia* and *Cystoisospora*, which was separated from *Isospora* to accommodate the facultatively heteroxenous *C. felis* and *C. rivolta* (Frenkel and Dubey 1972).

It is essential to accord generic distinction to obligate heteroxenous cyst-forming coccidia *Hammondia, Sarcocystis* and *Frenkelia*, because such organisms would never be propagated and studied experimentally, if the presence of obligate rather than facultative heteroxeny, as in *T. gondii*, were not recognized. Different host ranges, as between *H. hammondi, H. heydorni*, and *H. pardalis*, can be indicated by different species.

It took 129 years after the first observation of muscle cysts of *Sarcocystis* in a house mouse, and many unsuccessful attempts at homoxenous transmission, before an obligate heteroxenous cycle in *Sarcocystis* was recognized. This occurred first in *Sarcocystis* from sheep (separate species with cat or dog cycles), then in *Sarcocystis* from cattle (separate species and cycles through cat, dog, or human), thereafter in *Sarcocystis* from pigs (separate species through man or dog) (reviewed in Frenkel et al. 1979), and eventually in *Sarcocystis muris* from mice (Ruiz and Frenkel 1976).

The genera of tissue-cyst-forming, heteroxenous sarcocystid coccidia were re-defined (Frenkel 1977) and *Hammondia* was described with obligatory heteroxeny in verbal and tabular form, and compared with *Sarcocystis* and *Frenkelia*, although the designation "obligatory heteroxeny" was inadvertently omitted for *Hammondia*.

While one should not split genera extravagantly, there is no mandate in the Code of Zoological Nomenclature to be stingy in the designation of genera. Instead of maintaining a genus containing taxa that are too heterogeneous, it is practical to erect a new genus for its utilitarian value in characterizing certain differences. The several genera of cyst-forming isosporoid coccidia (Frenkel 1977) characterize practically important differences. Taxonomy, nomenclature, and phylogenetic analyses serve distinct purposes, although often there is a marked parallelism in their constructs.

Acknowledgement Original research supported by NIH grant AI-7489.

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